SOME ECOLOGICAL RELATIONSHIPS BETWEEN PHYTO- AND ZOOPLANKTON [1]

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The possibility of increasing human food resources by cultivation of unicellular algae is being rather widely investigated now (*cf.* Burlew, 1953). Two main approaches are considered: closed system cultivation of a pure algal culture under optimum growth conditions, and an open system, utilizing ponds. The closed system has the advantage of providing the maximal rate of photosynthesis and crop yield; however, it is expensive to install and maintain. The open system does not require elaborate installations and has the added advantage of utilizing ponds which may be constructed for a different purpose, algae being a by-product. Of such systems suitable for algal cultivation, sewage oxidation ponds are being studied. The growth of algae in these ponds is beneficial to the oxidation process, and the harvested algae save much of the nitrogen wastes and minerals which are otherwise poured into the sea (Gotaas *et al.*, 1954).

The solar evaporation ponds of the salt industry might also be utilized for this purpose. The natural productivity of these ponds per unit area approaches that of the open ocean (Carpelan, 1953), the difference being that the ponds are only one-half meter deep while the productive zone of the ocean is ten or more meters deep. This gives a very dense standing crop.

One of the economically limiting factors in mass cultivation of algae is their harvesting. In dense algal suspensions the volume of cells is still only a fraction of the total volume. To aid in the harvesting, suggested procedures have been sedimentation by slow settling (Smith, 1953) or flocculation of cells by added alum (Gotaas *et al.*, 1954).

Another possibility is utilizing grazing animals for the purpose of harvesting the algal cells. Raising fish in ponds is an old practice, the growth of plankton being accelerated by various fertilizers. However no attempt is usually made to maintain a maximal rate of production of the primary food, the algae.

In a preliminary investigation of the possibilities of the utilization of the sea water evaporation ponds of the salt industry, several unicellular algae which grow in this environment were studied (Gibor, 1956). We considered the possibilities of converting the algal crop to an animal crop by feeding a zooplankton grazer.

One of the important grazers in the evaporation ponds is the brine shrimp *Artemia*. This organism is easily maintained in the laboratory; its "eggs" (cysts) are readily available and can be kept in the laboratory for many years without losing their ability to hatch. We attempted to study the nutritive value to *Artemia* of several of the unicellular green algae which were isolated from the brines of the evaporation ponds. The algae used were:

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Dunaliella viridis
Dunaliella salina
Stephanoptera gracilis
Platymonas sp.
Stichococcus sp.

Dunaliella salina and *Stephanoptera gracilis* were cultured in sea water evaporated to half its original volume; the other three species were cultivated in sea water.

The feeding experiments were carried out as follows. *Artemia* cysts had been collected two years earlier from salt ponds of the Leslie Salt Company, and kept in a closed jar in the laboratory. Such cysts were suspended in water and centrifuged (in a clinical centrifuge) for several minutes. The light cysts were decanted off as suggested by Dempster (1953). The heavy cysts were re-suspended in merthiolate solution (1 : 1000, in water) for 10 minutes, then centrifuged. The merthiolate solution was decanted off and the cysts washed four times with sterile sea water to get rid of the merthiolate. Finally the sterile cysts were transferred into a one-liter Erlenmeyer flask containing sterile sea water and allowed to hatch.

The sterility of the cysts and larvae was determined by suspending a fraction of the sterilized cysts in sea water enriched with 2% yeast extract-glucose solution and

TABLE I

Size of Artemia salina fed on different brine algae for six days (average length exclusive of caudal furca)

Alga	Sterile culture	Non-sterile culture
Stephanoptera gracilis	2.3 mm.	2.8 mm.
Dunaliella viridis	2.1 mm.	2.8 mm.
D. salina	1.5 mm.	2.3 mm.
Platymonas sp.	1.5 mm.	2.0 mm.
Stichococcus sp.	0.7 mm.	0.4 mm.
None (unfed)	0.2 mm.	0.2 mm.

incubating for several weeks. To further verify their sterility a dense cyst suspension (⅓ ml. eggs in one ml. enriched sea water) was also incubated. The autolysing cysts should supply additional growth requirements for contaminating bacteria. Both these tests were negative and indicated the adequacy of the sterilization method. The larvae were transferred when needed with a sterile Pasteur pipette into a test tube for addition to the algal culture. Ten to 20 larvae were introduced into one liter of a dense algal culture in a two-liter Fernbach flask. The culture was kept under continuous illumination, with continuously bubbling air. After 6 days the cultures were still green, indicating that until this time the quantity of food was not limiting the growth of the animals. At this stage the *Artemia* were harvested by passing the whole solution through a fine plankton net. The animals were fixed in 0.5% formalin in sea water. The control larvae in sterile sea water were dead of starvation at this time.

The results of the feeding experiment are shown in Table I.

Dunaliella viridis and *Stephanoptera gracilis* appear to be superior as foods to *Dunaliella salina* and *Platymonas* sp., while *Stichococcus* sp., is evidently a poor nutrient for *Artemia*. Consistent results were obtained in a second experiment ex-

cept that growth on *Dunaliella salina* in this case was as good as on the other two Polyblepharidaceae.

For ecological purposes it seemed advisable to find whether these results also hold under non-sterile conditions. The apparent superiority of the Polyblepharidaceae might, for example, be due to the absence of a rigid cellulose wall; bacteria (in the *Artemia* gut) might aid in the digestion of the cellulose wall of *Platymonas* and *Stichococcus*. An experiment therefore was conducted under conditions identical to the first except for the use of unsterilized *Artemia* larvae. These results (Table I) show an improved growth in all cultures except *Stichococcus* sp. with the Polyblepharidaceae still showing better growth. Bond (1933) found that *Platymonas* sp. was slightly superior to *Dunaliella viridis* as food for *Artemia*. His criterion was the time in which *Artemia* reached the mating stage: on *Platymonas* sp. the required time was 28 to 29 days, on *Dunaliella viridis* 31 days. However in one of our experiments non-sterile *Artemia*, growing on *Dunaliella viridis*, were found copulating after 13 days.

To investigate whether *Stichococcus* is producing an inhibitor to the growth of *Artemia*, a mixed culture of *Stichococcus* sp. and *Dunaliella viridis* was inoculated with *Artemia* larvae. Good development of the larvae showed that no inhibitor was produced.

Microscopic observation established that the *Artemia* larvae do ingest *Stichococcus;* the deficient growth on this alga is thus not due to the inability of the animal to filter and ingest the smaller cells of this genus.

On the basis of the estimation of the *Stichococcus* crop and the population of *Artemia* in the evaporation ponds Carpelan (1953) concluded that *Artemia* utilizes only a small fraction of the crop of *Stichococcus*. Our results based on laboratory tests corroborate this opinion.

The observations made in the experiment on the nutritive value of algae for *Artemia* aid in understanding the ecological relationships in the series of evaporation ponds.

One of the striking facts observed in the salt ponds is the predominance of *Stichococcus* in brines of relatively low salinity (to about three-fold sea water). Both *Platymonas* sp. and *Dunaliella viridis* can be isolated from such low salinity brines, and both algae grow well in these concentrations. However, they are always overgrown by *Stichococcus*.

The finding that *Artemia* does not grow on *Stichococcus* suggested that the animal might act as a differential filter, ingesting the algae on which it grows well and leaving those on which it can not grow. The possibility of a differential mechanical effect was eliminated by observations on starved *Artemia* put into a *Stichococcus* suspension. As mentioned above, the animals fill their gut with this alga in a few minutes.

To determine whether live cells survive in the fecal pellets, sterile *Artemia* were fed on a pure culture of *Stichococcus*. After feeding for several hours the animals were washed by transferring them into a corner of a Petri dish containing sterile sea water. Use was made of the positive phototropic response of the *Artemia*. The fast swimming animals were collected from the opposite, light side of the dish and transferred to a second dish for repeated washing. After 4–5 such washings the animals were transferred into a depression slide containing sterile sea water and left for several hours. Fecal pellets accumulated in the depression slide. Single pel-

lets were picked with a sterile Pasteur pipette and transferred through a series of sterile sea water droplets. The washed pellets were finally inoculated into test tubes containing several milliliters of sea water enriched with minerals, and kept under continuous illumination.

Test tubes in which growth of algae occurred were examined microscopically to determine whether we were dealing with the same algae as fed to the animal. Ten test tubes so treated were found to contain growing *Stichococcus* cultures. Clearly some cells survived ingestion. An ecological advantage for one algal species over another might be established by even a slight difference in such ease of digestibility in the gut of a non-differentiating filter feeder.

In order to investigate this possibility in a mixed algal population the following experiment was performed. Young growing cultures of *Stichococcus, Dunaliella viridis* and a mixture of both algae were divided into two equal portions of 10 ml. each in 50-ml. Erlenmeyer flasks. Into one flask of each pair about 12 *Artemia* larvae were added. The flasks were kept under continuous illumination.

After ten days the following results were recorded: the *Stichococcus* flasks were both equally green, and growing. No appreciable growth of the larvae had occurred although some were still alive. The *Dunaliella viridis* cultures were entirely different. The flask without the animals was bright green, while the flask containing the animals was completely clear, and with the larvae growing well. The results of the mixed cultures were striking. The flask without the animals was deep green and growth of both algae was obvious. (The presence of *Dunaliella viridis* was easily ascertainable without a microscope since motile cells accumulated on the illuminated side of the flask.) The flask with the animals was not as green as the control flask and no obvious population of *Dunaliella viridis* could be seen by superficial observation. The animals in this culture were alive and growing, but they were not as large as the animals grown on the pure *Dunaliella viridis* culture. In the mixed culture without animals microscopic examination revealed the presence of a large number of both *Stichococcus* and *Dunaliella* cells. In the flask containing the animals, very few *Dunaliella* cells could be seen among the many *Stichococcus* cells. Later observations on the flasks, three weeks after the beginning of the experiment, revealed that the well developed *Artemia,* which had eaten and cleared the *Dunaliella viridis,* were dead, apparently of starvation. There was an indication of fresh growth of *Dunaliella viridis.* The animals were also dead in the dense *Stichococcus* sp. culture. On the mixed culture several living individuals were seen. However they were not as well developed as the animals which grow on the pure *Dunaliella viridis* culture.

A similar experiment was performed with a different zooplankton organism, the copepod, *Tigriopus.* The results were similar to those with *Artemia.* These animals could not utilize the *Stichococcus* cells available to them; *Dunaliella viridis* cells, on the other hand, were readily consumed. The ecological implications of these observations are of considerable importance. In standard oceanographic observations all the phytoplankton is considered as available food to the zooplankton. The curious phenomenon, often observed, of the scarcity of zooplankton in waters rich in phytoplankton, and vice-versa, was explained as due either to overgrazing (Harvey *et al.,* 1935) or to animal exclusion by production of inhibitors (Ryther, 1954). The present study suggests the possibility that certain phytoplankton organisms are not suitable food for some planktonic grazers.

Summary

1. Several planktonic algae from the brines of the sea water evaporation ponds were fed to the brine shrimp *Artemia*. They were found to differ in their nutritive value to this filter-feeding animal. One of these algae, *Stichococcus* sp., could not be utilized by the animal as a food source.

2. Controlled experiments of the effect of filter-feeding *Artemia* and *Tigriopus* on a mixed population of two unicellular algae indicate that the animals are capable of acting as differential grazers. The heavy bloom of *Stichococcus* in the evaporation ponds could be due to the effect of preferential digestion of competing algae by grazing animals.

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